Full text open access online (Since 2001)

PRINT: ISSN 0972-3757 ONLINE: ISSN 2456-6330
Int J Hum Genet, 24(4): 392-401 (2024)
DOI: 10.31901/24566322.2024/24.04.896

Tomentin-A Targets MMP and Ras/Raf/MEK/ERK Pathway in Cisplatin-Resistant Lung Cancer Cells to Induce Oxidative Stress Mediated Apoptosis

Bin Yang[1], Fengchang Zheng[1], Weilin Yang[1], Peng Sun[1], Hailong Zhao[1] and Xiaokang Zhu[1]

[1]Department of Thoracic Surgery, Gansu Provincial Cancer Hospital, No.2, Xiaoxihu East Street, Qilihe District, Lanzhou, Gansu, 730050, China

KEYWORDS Antiproliferative. Apoptosis. Tomentin-A. Lung Cancer. Mitochondrial Membrane Potential. Reactive Oxygen Species

ABSTRACT The current study investigated Tomentin-A as an inhibitory agent against lung cancer cells. Tomentin-inducing antiproliferative effects on the A549 cisplatin-resistant NSCLC line were examined using an MTT test, revealing cell colonies through microscopy. Apoptosis-inducing activity was assessed via DAPI labelling and Western blotting, while intracellular-ROS and mitochondrial-membrane-potential were measured using DCFH-DA and Rh-123 assays. Migration and invasion were evaluated using transwell assays, and Western blotting explored Tomentin-A's regulation of the Ras/Raf/MEK/ERK pathway. Results showed that Tomentin substantially ($p<0.05$) suppressed A549 drug-resistant NSCLC cell growth and targeted colonies dose-dependently. DNA condensation and fragmentation were observed in nuclear morphology after DAPI labelling, while ROS and MMP assays indicated oxidative stress-induced cell death. Migration and invasion investigations demonstrated Tomentin's strong inhibitory effects, and Western blotting showed considerable downregulation of phosphorylated Ras, Raf, MEK, and ERK. These findings highlight Tomentin's potential as a multifunctional treatment for drug-resistant NSCLC, warranting further clinical investigation.

INTRODUCTION

Cancer remains a substantial challenge to human well-being, and lung cancer is particularly noteworthy for its impact on morbidity and mortality on a global scale. While chemotherapeutic agents, such as cisplatin, have significantly improved treatment outcomes (Chhikara and Parang 2023; Siegel et al. 2023), the persistent challenge of drug resistance poses a substantial hurdle in sustaining long-term therapeutic efficacy. In this intricate environment, there has been a growing focus on natural compounds as possible solutions to combat drug resistance and strengthen the fight against cancer (Khursheed et al. 2021; Khursheed and Jain 2021). Tomentin-A, a compound derived from natural sources, has gained attention for its potential therapeutic properties.

The intricate interplay between carcinoma cells and their neighbouring milieu, combined with the ability of cancerous populations to adapt, leads to the emergence of resistance against chemotherapy drugs. Cisplatin, a crucial component to target lung cancer, highlights the ongoing issue of drug resistance. This calls for the need to explore alternative approaches to overcome this significant hurdle. In the pursuit of new therapeutic possibilities, Tomentin-A has emerged as a promising contender, displaying distinctive qualities that warrant in-depth exploration.

Sphaeralcea angustifolia, commonly known as "vara de San José" in Mexico, belongs to the Malvaceae family (Pérez-Hernández et al. 2019; Meckes-Fischer and Nicasio-Torres 2023). Ethnobotanical knowledge highlights its traditional use in treating inflammatory processes and wound healing. The CH_2Cl_2 extract from the plant's aerial parts has shown notable anti-inflammatory activity in mice and rats during acute and chronic phases, with evidence of cytokine regulation (Reyes-Pérez et al. 2022). Scopoletin was pinpointed as the bioactive compound, alongside the later discovery of the monoterpene loliolide in the methanolic extract. Despite its endangered status in Mexico, *Sphaeralcea angustifolia* continues to be valued for its medicinal properties, particularly its anti-inflammatory effects. Recent research on suspension-cultured cells isolated two compounds, tomentin and sphaeralcic acid, both demonstrating anti-inflammatory properties (Serrano-Román et al.

Address for correspondence:
Xiaokang Zhu[1]
E-mail: zhuxiaokang_2006@hotmail.com

2020). Tomentin inhibited footpad edema by 58 percent, while sphaeralcic acid achieved a 66 percent inhibition at a dose of 45 mg/kg. Local application of such compounds also exhibited significant inhibitory effects on auricular edema formation, underscoring their potential therapeutic significance in addressing inflammatory conditions.

Oxidative stress in cancer is a complex, extensively researched phenomenon involving a disruption in the equilibrium between the generation of ROS and the body's ability to effectively detoxify or repair the ensuing damage (Hayes et al. 2020; Arfin et al. 2021). Elevated ROS levels induce oxidative stress, contributing to DNA mutations, protein damage, and lipid peroxidation, crucial in cancer initiation and progression. Chronic oxidative stress promotes inflammation, activates signalling pathways supporting cancer cell survival and proliferation, while compromising immune surveillance (Jelic et al. 2021; Alhamzah et al. 2023). Additionally, oxidative stress is implicated in angiogenesis, metastasis, and apoptosis resistance, key in tumour development. Understanding this intricate interplay has spurred ongoing research for antioxidant-based therapeutic strategies to effectively mitigate oxidative damage, presenting potential avenues for cancer prevention and treatment.

Apoptosis, a fundamental cellular process, entails programmed cell death crucial for tissue homeostasis and the removal of damaged cells. This highly regulated mechanism involves caspase activation, DNA fragmentation, and membrane blebbing. Playing a pivotal role in embryonic development, immune system regulation, and cancer defence, apoptosis ensures controlled elimination, preventing uncontrolled proliferation and maintaining cellular health (Carneiro and El-Deiry 2020; Gao et al. 2020; Singh and Lim 2022). The Ras/Raf/MEK/ERK pathway serves as a pivotal signalling cascade that governs various cellular processes, such as survival, differentiation, and growth. Triggered by environmental cues, such as growth factors, it sequentially activates Ras, Raf, MEK, and ERK. Dysregulation, notably in cancer, leads to uncontrolled proliferation (Degirmenci et al. 2020). A prime therapeutic target, interventions aim to modulate aberrant signalling, halting disease progression. Crucial for cellular bioenergetics and apoptosis regulation, the MMP is an internal mitochondrial electrochemical gradient. Generated during electron transport, disruption is an early apoptosis hallmark, triggering pro-apoptotic factor release. Reflecting mitochondrial health, changes impact cellular energy production, crucial in understanding the intricate balance between cell survival and death. Monitoring this potential provides insights into stress responses and apoptosis (Gao et al. 2020).

Objectives

This in-depth analysis seeks to uncover the complex effects of Tomentin-A on lung cancer cells that are resistant to cisplatin. Through an in-depth exploration of the complex molecular mechanisms that drive its antiproliferative effects, the goal is to offer a comprehensive understanding of the compound's prospective as a phytopharmaceutical agent. With its ability to induce oxidative stress and modulate critical cellular pathways, Tomentin-A emerges as a highly versatile candidate worthy of further investigation. Understanding its impact on apoptosis, migration, invasion, and MMP activity enhances its potential uses in cancer treatment.

MATERIAL AND METHODS

Cell Culture

The A549 cell line, a model for cisplatin-resistant non-small cell lung cancer (NSCLC), was acquired from the American Type Culture Collection (USA) and cultivated in RPMI-1640 medium supplemented with 10 percent FBS and penicillin/streptomycin (p/s) at 100 units/ml. The cells were maintained at 37°C in a controlled humidified atmosphere with 5 percent CO_2. To assess the impact of Tomentin-A, a chemical of interest, it was precisely dissolved in DMSO at 0 to 160 μM. The resulting Tomentin-A solutions were methodically administered to the cultured cells during an incubation period of either 24 or 48 hours, in accordance with experimental design and stringent adherence to established protocols.

MTT Assay

A549 cells (5,000 cells per well) were seeded into a 96-well plate a day prior to the initiation of Tomentin treatment. Subsequent to the treatment, cellular proliferation was facilitated for either 24 or 48 hours. Following this incubation period with Tomentin, MTT (0.5 mg/ml) was introduced to the

wells and incubated at 37°C for 1 hour. Subsequently, the formazan crystals formed were dissolved with 100 µl of DMSO. The samples were assessed for absorbance at 570 nm using a Multiskan GO spectrophotometer from Thermo Scientific (Finland). In the assessment of relative cell viability, reference was made to the negative control containing 0 µM Tomentin-A in DMSO.

Clonogenic Assay

For the clonogenic assay, A549 cells (1,000 cells/well) were initially plated into six-well plates one day before treatment with Tomentin. Following treatment, the cultures were allowed to proliferate for 10-14 days. For visualisation and manual counting, colonies were fixed and stained with methanol and crystal violet, respectively. After counting colonies with 50 cells or more, the surviving percentage was determined by comparing it to the control group that was left untreated. The assay was performed in triplicate, and the results were analysed to determine the clonogenic potential of A549 cells following Tomentin treatment.

DAPI Staining

DAPI staining was employed to assess apoptosis in A549 cells following exposure to Tomentin. The A549 cells were treated with Tomentin for 48 hours at concentrations of 0, 20, 80, and 160 µM. After the 48-hour incubation period, cells were washed with PBS and fixed at 4°C in a 3:1 methanol/acetic acid fixing solution. For nuclear visualisation, cells were initially rinsed with PBS and subsequently stained in the dark for 30 minutes with a 10 µmol/L working solution of DAPI. After the treatment period, cells were washed with PBS, and apoptotic nuclear morphological changes were observed under a fluorescence microscope. Additionally, bright-field microscopy was utilised to investigate morphological alterations following exposure to Tomentin. This assay was conducted in triplicate to assess the impact of Tomentin on nuclear morphology and potential apoptotic changes in A549 cells.

ROS Estimation

The DCFH-DA assay was employed for assessment of the production of reactive oxygen species (ROS) as indicative marker of apoptosis in A549 cells administered with Tomentin-A. 1×10^5 cells were seeded to six-well plate for incubation overnight to establish their morphological characteristics. Subsequently, the cells were exposed to Tomentin at concentrations of 0, 20, 80, and 160 µM. Following a 48-hour incubation period, PBS was utilised for cell washing, and 10 µmol/L DCFDA dye solution was added for a 20-minute incubation. After another round of PBS washing, fluorescent images were recorded with a fluorescence microscope to visualise the ROS levels. This assay, performed in triplicate for assessment of Tomentin on ROS production, a key indicator of apoptosis, in A549 cells.

MMP Assessment

Mitochondrial membrane potential was assessed using Rh123 staining in the context of this study. A549 cells, subjected to varying concentrations of Tomentin (0, 20, 80, and 160 µM) for 48 hours, were harvested, washed, and incubated with 10 µM Rh123 for 30 minutes at 37°C in the absence of light. The mean values from triplicate experiments were subsequently calculated, providing insights into the impact of Tomentin-A on mitochondrial membrane potential in A549 cells.

Migration and Invasion Assays were Conducted to Assess the Impact of Tomentin on A549 Cells

For the migration assay a modified Boyden chamber with an 8-µm pore size was employed. The upper chamber in a 24-well Transwell insert was filled with a total of 1.0×10^5 A549 cells suspended in 100 µl serum-free medium. At the same time, 600 µl culture medium (DMEM) with 10 percent FBS, as well as different amounts of Tomentin at various concentrations (0, 20, 40 and 160 µM), were put into the lower chamber. After that, for 48 hours, the cells were kept at 37°C. The cells that had migrated to the bottom of the Transwell were then fixed in 100 percent methanol and stained with 0.5 percent crystal violet for 15 minutes at room temperature to get the desired hue. Images of migrated cells in three randomly chosen fields were collected and examined under a light microscope after non-migrated cells on the top surface were obliterated.

A 24-well Transwell plate was prepared for the invasion experiment by applying Matrigel (BD Biosciences) to the top chamber and then incubating it at 37°C to polymerize it. There was 600 µl of DMEM medium with 10 percent FBS in the bottom chamber. Introduced into the top chamber were

A549 cells (1.0×10^5) in 100 µl serum-free media, which were then treated with Tomentin at different doses. Similar to the migration experiment, the invasion assay was carried out after a 48-hour incubation period at 37°C. The rate of invasion inhibition was determined by counting invading cells in three replicas for each well.

Western Blotting

To reveal the protein-level molecular consequences of Tomentin on the Ras/Raf/MEK/ERK pathway, Western blot analysis was performed on A549 cells. Total proteins from A549 cells treated with Tomentin were extracted using a lysis buffer. The targeted proteins, including Ras, phosphorylated Ras (p-Ras), Raf, phosphorylated Raf (p-Raf), MEK, phosphorylated MEK (p-MEK), ERK, and phosphorylated ERK (p-ERK), were separated using denaturing SDS-PAGE and with subsequent transfer onto a PVDF membrane. Following transfer, the PVDF membrane was blocked and incubated with antibodies specific to p-Ras, Ras, p-Raf, Raf, p-MEK, MEK, p-ERK and ERK. Subsequently, secondary antibody incubation was performed, and visualising the proteins of interest was achieved through the application of an enhanced chemiluminescence kit. The chemiluminescent signals were then detected using a chemiluminescence analyser. This targeted analysis aimed to reveal alterations in the levels of p-Ras, Ras, p-Raf, Raf, p-MEK, MEK, p-ERK and ERK in response to Tomentin treatment in A549 cells.

Statistical Analysis

Experimental data was statistically analysed using GraphPad software. Results were presented as mean ± standard deviation and group comparisons using the Student's t-test, which indicated statistical significance between groups with a two-tailed significance set at $P<0.05$.

RESULTS

Targeting of Proliferation and Colony Formation by Tomentin

The outcomes of the MTT assay indicated how Tomentin influenced the viability of A549 cells during both 24-hour and 48-hour intervals. After 24 hours of treatment, the cell viability exhibited a concentration-dependent decline, with percentages decreasing from 97 percent (control) to 16 percent at 160 ìM. This trend intensified after 48 hours, where the control viability remained high at 92 percent, but the viability at 160 ìM precipitously dropped to 4 percent as evident from Figure 1. The observed decrease in cell viability signifies a time-dependent and dose-dependent effect of Tomentin, suggesting its potential influence on A549 cell survival and proliferation. These findings underscore the importance of considering both concentration and exposure duration in assessing the impact of Tomentin on cellular viability in the context of A549 cells.

The clonogenic assay reveals a discernible dose-dependent inhibition of colony formation in A549 cells following Tomentin treatment. The observed reduction in colony formation in Figure 2 becomes increasingly evident with ascending concentrations of Tomentin (0, 20, 80, and 160 µM). This underscores the compound's capacity to influence and impede the ability of A549 cells to form colonies within this specific timeframe, highlighting the significance of concentration in modulating clonogenic potential.

Apoptosis Induction

The DAPI staining assay provided detailed insights into the cellular response to Tomentin A treatment. The results showcased in Figure 3 report notable phenomena of nuclear disintegration including DNA fragmentation and condensation, indicating the induction of apoptosis. Importantly, this effect manifested in a manner dependent on the administered dose, suggesting that higher concentrations of Tomentin A led to a more pronounced and widespread apoptotic response. The DAPI staining, which specifically binds to DNA and highlights nuclear morphology, served as a reliable marker for apoptosis induction. The findings underscore the potential of Tomentin A to modulate cellular processes, particularly apoptosis, and highlight the importance of dosage in influencing its impact on nuclear integrity and cell fate.

Targeting of Migration and Invasion by Tomentin

The results from both the Transwell migration and invasion assays, particularly with the incorporation of Matrigel in the invasion assay, dis-

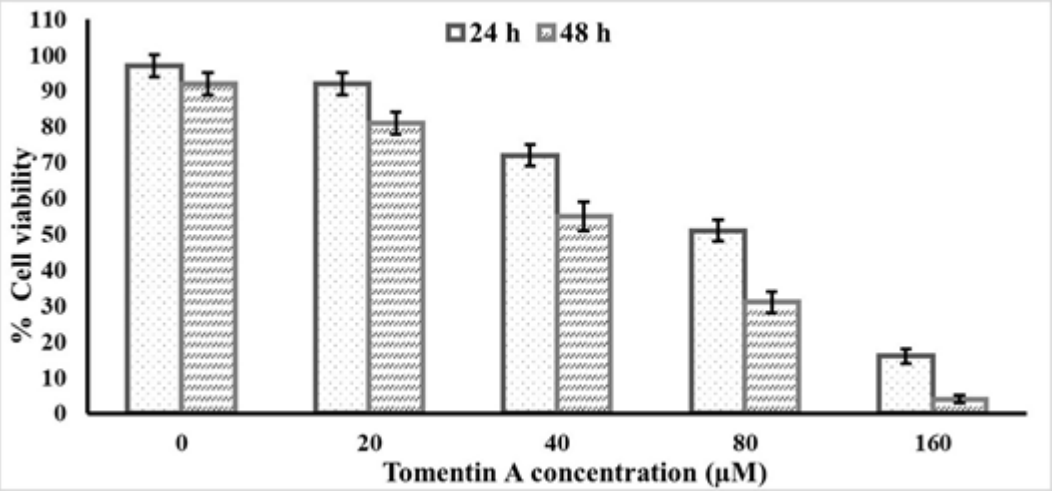

Fig. 1. Effects of Tomentin on A549 Cell Viability: The MTT assay results depict the influence of Tomentin on the viability of A549 cells over 24 and 48 hours. Following 24 hours of treatment, a concentration-dependent decline in cell viability is evident, with percentages decreasing from 97% (control) to 16% at 160 ìM. This decline intensifies after 48 hours, as the control viability remains high at 92%, contrasting sharply with the viability at 160 ìM, which precipitously drops to 4% ($p<0.05$)

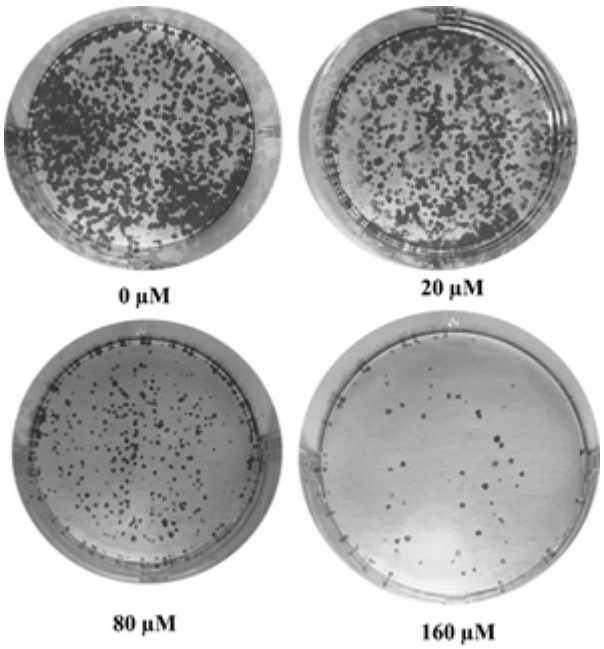

Fig. 2. The clonogenic assay demonstrates a noticeable dose-dependent inhibition of colony formation in A549 cells upon Tomentin treatment. The discernible reduction in colony formation becomes increasingly evident with ascending concentrations of Tomentin (0, 20, 80, and 160 ìM)

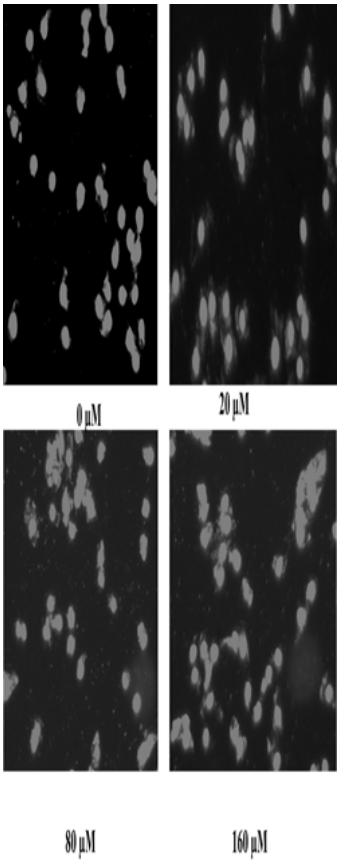

Fig. 3. Tomentin A-induced Apoptosis: DAPI staining assay reveals dose-dependent nuclear disintegration, DNA fragmentation, and chromatin condensation in A549 cells treated with Tomentin A at concentrations of 0, 20, 80 and 160 µM

tinctly demonstrate the dose and time-dependent inhibition exerted by Tomentin on the migratory capabilities and invasive potential of A549 cells.

In the Transwell migration assay conducted for 48 hours, the number of migrated cells decreased progressively with increasing concentrations of Tomentin-A (0, 20, 80, and 160 µM) as illustrated from Figure 4. This observation supports the notion that Tomentin induces a dose-dependent inhibition of cell migration. Moreover, in the invasion assay, where Matrigel was utilised to mimic a more complex extracellular matrix, Tomentin exhibited a dose-dependent reduction in the number of invading cells after 48 hours. The inhibitory effect

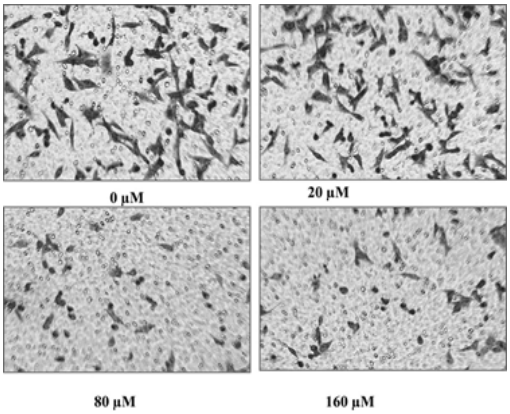

Fig. 4. Tomentin-induced Inhibition of Cell Migration: In the 48-hour Transwell migration assay, the number of migrated cells progressively decreases with increasing concentrations of Tomentin (0, 20, 80, and 160 ìM). This observation supports the concept that Tomentin induces a dose-dependent inhibition of cell migration

on invasion reported in Figure 5 becomes more apparent with higher concentrations of Tomentin (0, 20, 80, and 160 ìM), indicating a significant impediment to the invasive capacity of A549 cells.

These findings collectively underscore the ability of Tomentin to dose and time-dependently inhibit both migration and invasion of A549 cells, revealing its potential as a modulator of these critical cellular processes.

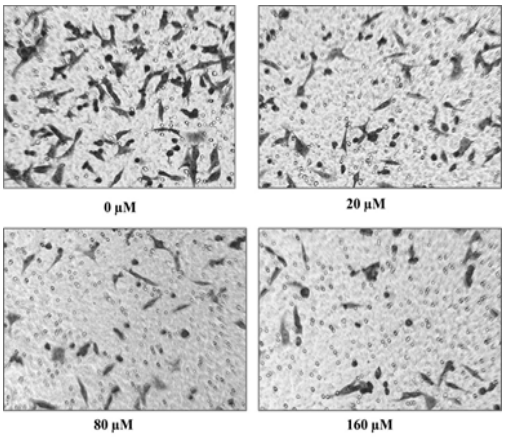

Fig. 5. Tomentin Inhibits Cell Invasion: Tomentin shows a dose-dependent reduction in invaded cells in a 48-hour invasion assay using Matrigel. Higher concentrations (0, 20, 80, and 160 ìM) significantly impede A549 cell invasion

Targeting of ROS Formation by Tomentin

The DCFH-DA assay in A549 cells demonstrated a dose and time-dependent elevation in ROS production induced by Tomentin. A total of 1×10^5 cells were treated with Tomentin concentrations (0, 20, 80, and 160 μM) for 48 hours. Following incubation, DCFDA dye revealed increasing fluorescence as shown in Figure 6, indicative of rising ROS levels, with higher Tomentin concentrations. Images captured using a fluorescence microscope portrayed a clear, concentration-dependent enhancement in ROS production. The assay, conducted in triplicate, provides robust evidence of Tomentin's influence on cellular oxidative stress, further emphasising its potential role in modulating apoptosis-related pathways in A549 cells.

Targeting of MMP by Tomentin

The assessment of MMP through Rh123 staining reveals a clear and dose-dependent impact of Tomentin on MMP loss in A549 cells. Cells exposed to Tomentin concentrations (0, 20, 80, and 160 μM) for 48 hours exhibited a gradual decline in MMP, as indicated by reduced Rh123 staining. The quantification of mean values from triplicate experiments demonstrated a notable and dose-dependent decrease in MMP levels, illustrated in Figure 7, with increasing concentrations of Tomentin. This outcome underscores the compound's ability to induce time and dose-dependent disruption of mitochondrial membrane potential in A549 cells, suggesting a potential role in influencing mitochondrial function and cellular homeostasis.

Targeting of Ras/Raf/MEK/ERK Pathway by Tomentin

The Western blot analysis of A549 cells treated with Tomentin provided insights into the molecular impact on the Ras/Raf/MEK/ERK pathway at the protein level. The results demonstrated in Figure 8, show a distinctive pattern in the expression of phosphorylated proteins compared to their non-phosphorylated counterparts. Specifically, Ras, Raf, MEK, and ERK proteins exhibited consistent expression levels, indicating minimal change in their non-phosphorylated states in response to Tomentin treatment. Conversely, the phosphorylated forms displayed a discernible increase in expression, highlighting a clear upregulation of phosphorylation events in the pathway. This targeted analysis effectively underscores Tomentin's im-

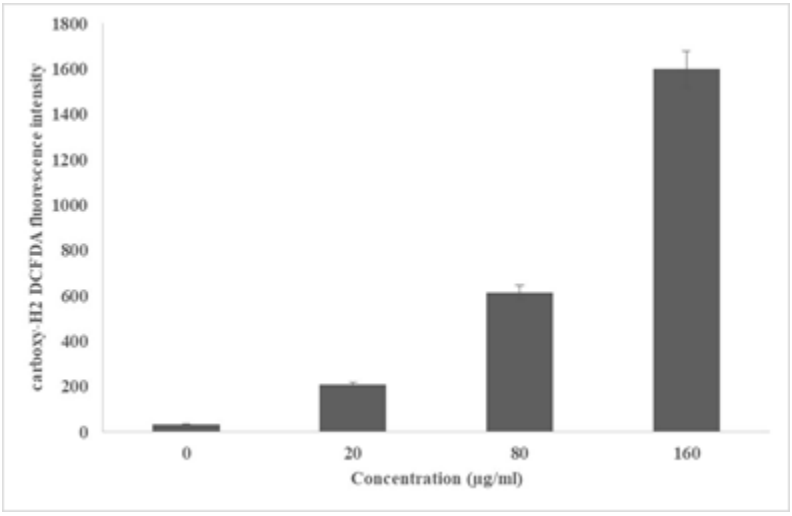

Fig. 6. Tomentin Induces ROS in A549 Cells: Tomentin treatment (0, 20, 80, and 160 ìM) for 48 hours shows a dose and time-dependent increase in ROS levels in A549 cells, as evidenced by DCFDA dye fluorescence. Fluorescence microscopy confirms a concentration-dependent rise in ROS production, emphasizing Tomentin's impact on cellular oxidative stress. (*p<0.05)*

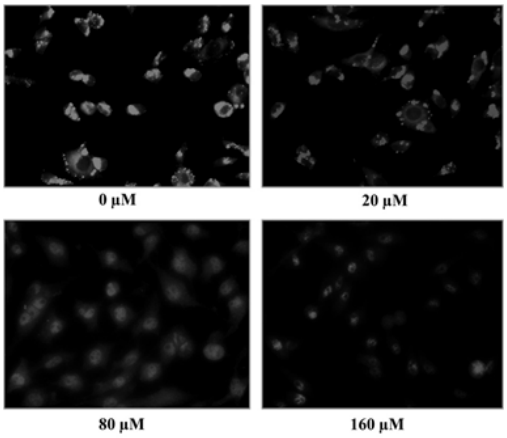

Fig. 7. Decreased fluorescence intensity of tomentin-A treated cells indicative of loss of mitochondrial membrane potential in cancer cells with increase in the concentration of the tested molecule. Rh-123 staining dye was used as a staining agent and images were captured by a fluorescence microscope

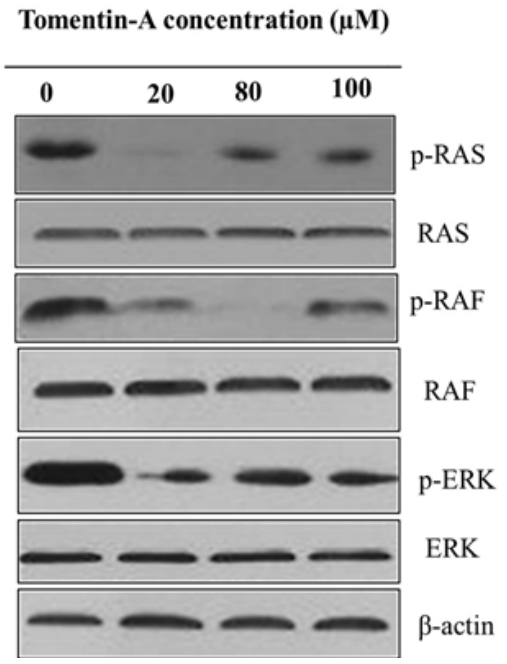

Fig. 8. Tomentin-A Modulates Phosphorylation in Ras/Raf/MEK/ERK Pathway: Western blot analysis reveals Tomentin-A induced upregulation of phosphorylated forms (p-Ras, p-Raf, p-MEK, p-ERK) in the Ras/Raf/MEK/ERK pathway, highlighting selective modulation of phosphorylated components

pact on the activation of key signalling proteins through enhanced phosphorylation, suggesting a selective modulation of the phosphorylated components within the pathway while leaving the non-phosphorylated forms relatively unaffected.

DISCUSSION

The comprehensive analysis of Tomentin's effects on A549 lung cancer cells provides valuable insights into its multifaceted impact on key cellular processes. The study employed a variety of assays to investigate Tomentin's influence on cell viability, clonogenic potential, apoptosis induction, migration, invasion, ROS formation, MMP, and the Ras/Raf/MEK/ERK pathway. The results, considered in the context of existing literature, offer a nuanced understanding of Tomentin's potential as a modulator of cancer-related pathways.

The substantial interest in natural products owing to their propensity of combating the proliferation of carcinoma cells has led this research on Tomentin to align with this broader goal. An abundance of naturally derived compounds sourced from a diverse array of organisms, encompassing plants, fungi, and marine organisms, have demonstrated anti-proliferative effects on cancer cells (Mukherjee et al. 2001; Siu 2011; Deng et al. 2020). These bioactive molecules often interfere with significant cellular processes, comprising DNA synthesis, cell cycle regulation, and mitosis, ultimately leading to the inhibition of cancer cell proliferation (Tewari et al. 2022; Sajadimajd et al. 2020). The diverse chemical structures of natural products contribute to their versatility in targeting different molecular pathways involved in cancer cell growth. Herein, the MTT assay revealed a time and dose-dependent impact of Tomentin on A549 cell viability. After 24 hours, a concentration-dependent decline in viability was evident, further intensifying after 48 hours. This aligns with existing literature suggesting the importance of exposure duration and compound concentration in influencing cell survival and proliferation. The observed decrease in viability underscores Tomentin's potential as an antiproliferative agent. The clonogenic assay reinforced the MTT findings, emphasising Tomentin's capacity to dose-dependently inhibit colony formation in A549 cells. This corroborates literature indicating the significance of clonogenic assays in assessing the long-term impact of treatments on

cell survival and proliferation (Adan et al. 2016). Tomentin's ability to modulate clonogenic potential further supports its potential as an anti-cancer agent. The DAPI staining assay elucidated Tomentin's role in apoptosis induction. The observed nuclear alterations, including DNA fragmentation and condensation, highlight Tomentin's pro-apoptotic effects in a dose-dependent manner. This aligns with literature suggesting that compounds inducing nuclear changes may trigger apoptosis-related pathways, indicating Tomentin's potential as an apoptosis-modulating agent (Wang et al. 2020). The findings demonstrated Tomentin's dose and time-dependent inhibition of A549 cell migration and invasion. These findings align with literature emphasising the importance of assessing both migration and invasion to comprehensively understand a compound's anti-metastatic potential (Abdullah et al. 2021). Tomentin's inhibitory effects on migration and invasion suggest its potential as a metastasis-modulating agent. The DCFH-DA assay provided evidence of Tomentin-induced ROS production in a manner dependent on the administered dose. Increased ROS levels are often associated with apoptosis induction, supporting the DAPI staining results (Gao et al. 2020). Literature supports the link between ROS generation and apoptosis, further highlighting Tomentin's potential in modulating apoptosis-related pathways through oxidative stress (Slika et al. 2022). The Rh123 staining assay demonstrated Tomentin's dose-dependent impact on MMP loss in A549 cells. The disruption of mitochondrial membrane potential suggests potential effects on mitochondrial function and cellular homeostasis (Liao et al. 2021). Existing literature supports the significance of MMP loss in apoptotic processes, indicating Tomentin's potential in modulating mitochondrial integrity (Hinz and Lagares 2020). The Western blot analysis of the Ras/Raf/MEK/ERK pathway revealed a selective impact of Tomentin on phosphorylated proteins. While non-phosphorylated proteins showed minimal changes, phosphorylated forms exhibited increased expression, indicating enhanced phosphorylation events. Literature supports the role of phosphorylation in signalling pathways, suggesting that Tomentin may selectively modulate specific components within the Ras/Raf/MEK/ERK pathway.

CONCLUSION

In conclusion, the study provides a comprehensive exploration of Tomentin's effects on A549 cells, shedding light on its potential as a modulator of crucial cellular processes. The observed time and dose-dependent impacts on viability, clonogenic potential, apoptosis induction, migration, invasion, ROS production, MMP loss, and the Ras/Raf/MEK/ERK pathway align with existing literature, supporting further investigation into Tomentin's therapeutic potential in lung cancer treatment.

RECOMMENDATIONS

The study's findings contribute valuable information to the growing body of research on natural compounds with anti-cancer properties and suggested Tomentin A as a multifaceted therapeutic agent against drug-resistant NSCLC, warranting further exploration of its clinical applications.

FUNDING

Not applicable.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest to indicate.

REFERENCES

Abdullah NA, Md Hashim NF, Ammar A, Muhamad Zakuan N 2021. An insight into the anti-angiogenic and anti-metastatic effects of oridonin: Current knowledge and future potential. *Molecules,* 26(4): 775.

Adan A, Kiraz Y, Baran Y 2016. Cell proliferation and cytotoxicity assays. *Curr Pharm Biotechnol,* 17(14): 1213-1221.

Alhamzah SA, Gatar OM, Alruwaili NW 2023. Effects of ketogenic diet on oxidative stress and cancer: A literature review. *Adv Cancer Biol: Metastasis*, 100 093.

Arfin S, Jha NK, Jha SK, Kesari KK, Ruokolainen J et al. 2021. Oxidative stress in cancer cell metabolism. *Antioxidants,* 10(5): 642.

Carneiro BA, El-Deiry WS 2020. Targeting apoptosis in cancer therapy. *Nat Rev Clin Oncol*, 17(7): 395-417.

Chhikara BS, Parang K 2023. Global Cancer Statistics 2022: The trends projection analysis. *Chem Biol Lett*, 10(1): 451.

Degirmenci U, Wang M, Hu J 2020. Targeting aberrant RAS/RAF/MEK/ERK signaling for cancer therapy. *Cells*, 9(1): 198.

Deng LJ, Qi M, Li N, Lei YH et al. 2020. Natural products and their derivatives: Promising modulators of tumor immunotherapy. *J Leukoc Biol*, 108(2): 493-508.
Gao L, Loveless J, Shay C, Teng Y 2020. Targeting ROS-mediated crosstalk between autophagy and apoptosis in cancer. *Reviews on New Drug Targets in Age-related Disorders*, 1-2.
Hayes JD, Dinkova-Kostova AT, Tew KD 2020. Oxidative stress in cancer. *Cancer Cell*, 38(2): 167-197.
Hinz B, Lagares D 2020. Evasion of apoptosis by myofibroblasts: A hallmark of fibrotic diseases. *Nat Rev Rheumatol*, 16(1): 11-31.
Jelic MD, Mandic AD, Maricic SM, Srdjenovic BU 2021. Oxidative stress and its role in cancer. *J Cancer Res Ther*, 17(1): 22-28.
Khursheed A, Jain V, Rasool A, Rather MA et al. 2021. Molecular scaffolds from mother nature as possible lead compounds in drug design and discovery against coronaviruses: A landscape analysis of published literature and molecular docking studies. *Microb Pathog*, 157: 104933.
Khursheed A, Jain V 2021. Phytochemical screening, antioxidant, and antimicrobial activity of different Portulaca oleracea L. extracts growing in Kashmir Valley. *J Biochem Technol*, 12(3): 1-8.
Liao HY, Da CM, Liao B, Zhang HH 2021. Roles of matrix metalloproteinase-7 (MMP-7) in cancer. *Clin Biochem*, 92: 9-18.
Meckes-Fischer M, Nicasio-Torres P 2023. The journey of a medicinal plant throughout science: Sphaeralcea angustifolia (Cav.) G. Don (Malvaceae). *Plants*, 12(2): 321.
Mukherjee AK, Basu S, Sarkar N, Ghosh AC 2001. Advances in cancer therapy with plant based natural products. *Curr Med Chem*, 8(12): 1467-1486.
Pérez-Hernández J, Martínez-Trujillo A, Nicasio-Torres P 2019. Optimization of active compounds production by interaction between nitrate and copper in Sphaeralcea angustifolia cell suspension using Response Surface Methodology. *Plant Cell Tissue Org Cult*, 136: 407-413.
Reyes-Pérez R, Herrera-Ruiz M, Perea-Arango I, Martínez-Morales F et al. 2022. Anti-inflammatory compounds produced in hairy roots culture of Sphaeralcea angustifolia. *Plant Cell Tissue Org Cult*, 149(1-2): 351-361.
Sajadimajd S, Bahramsoltani R, Iranpanah A, Patra JK et al. 2020. Advances on natural polyphenols as anticancer agents for skin cancer. *Pharmacol Res*, 151: 104584.
Serrano-Román J, Nicasio-Torres P, Hernández-Pérez E, Jiménez-Ferrer E 2020. Elimination pharmacokinetics of sphaeralcic acid, tomentin and scopoletin mixture from a standardized fraction of Sphaeralcea angustifolia (Cav.) G. Don orally administered. *J Pharm Biomed Anal*, 183: 113143.
Siegel RL, Miller KD, Wagle NS, Jemal A 2023. Cancer statistics, 2023. *Ca Cancer J Clin*, 73(1): 17-48.
Singh P, Lim B 2022. Targeting apoptosis in cancer. *Curr Oncol Rep*, 24(3): 273-284.
Siu D 2011 Sep. Natural products and their role in cancer therapy. *Med Oncol*, 28(3): 888-900.
Slika H, Mansour H, Wehbe N, Nasser SA et al. 2022. Therapeutic potential of flavonoids in cancer: ROS-mediated mechanisms. *Biomed Pharmacother*, 146: 112442.
Tewari D, Patni P, Bishayee A, Sah AN, Bishayee A 2022. Natural products targeting the PI3K-Akt-mTOR signaling pathway in cancer: A novel therapeutic strategy. *Semin Cancer Biol*, 80: 1-17.
Wang X, Hu Y, Mo J, Zhang J et al. 2020. Arsenene: A potential therapeutic agent for acute promyelocytic leukaemia cells by acting on nuclear proteins. *Angew Chem*, 132(13): 5189-5196.

Paper received for publication in
Paper accepted for publication in